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(54) [Title of the invention]

ANTI-TENASCIN C MONOCLONAL ANTIBODY AND HYBRIDOMA PRODUCING THE SAME

(57) [Abstract]

[Problem to be solved]

To provide a monoclonal antibody that recognizes the splicing variants specifically expressed in cancer tissues and inflammation tissues thereby contributing to a new therapeutic method for cancers and developing the medical materials effective for diagnosis of cancers and inflammations. [Solution]

Objective monoclonal antibody and hybridoma cells for producing the said antibody are provided by using the cDNA of tenascin-C splicing variant that is specifically expressed in cancer tissues or inflammation tissues to prepare the fused protein of the said variant. The monoclonal antibody according to the present invention is useful for diagnosing cancers and inflammations.

[Claims]
[Claim 1] An anti-tenascin C monoclonal antibody selectively recognizing tenascin-C splicing variant that is specific to cancer stroma.
[Claim 2] A hybridoma cell, FERM P-18070, having a function of producing the monoclonal antibody of claim 1.
[Claim 3] A hybridoma cell, FERM P-18173, having a function of producing the monoclonal antibody of claim 1.
[Claim 4] A diagnostic agent characterized in that the monoclonal antibody of claim 1 is labeled.
[Claim 5] A diagnostic agent kit characterized in that it comprises at least both elements of labeled anti tenascin-C secondary antibody for detecting tenascin-C binding to a plate with anti tenascin-C primary antibody fixed thereon as well as to the said primary antibody, and at least one of the said primary antibody or the said secondary antibody is the monoclonal antibody of claim 1.
[Claim 6] A method of detecting splicing variant that is specific to cancer stroma of the said tenascin C using the monoclonal antibody of claim 1.
[Detailed description of the invention]
[0001]
[Technical field of the invention]
The present invention relates to an anti-tenascin C monoclonal antibody and hybridoma cells for producing anti-tenascin C monoclonal antibody More specifically, it relates to an anti-tenascin C monoclonal and its producing cells which recognize the splicing variants of tenascin C specifically expressed in stroma of cancer tissues as well as reconstructing tissues thereby and capable of diagnosing cancers and inflammations or selective administration of anticancer drug.
[0002]
[Prior art]
Tenascin C (TN-C) is a kind of extracellular matrix glycoproteins wherein 3 molecules of polypeptide having an approximately 250000 molecular weight are coiled near N-terminal and further forming a hexamer by 2 disulfide bonds. Each polypeptide chain includes edidermal growth factor (EGF) -like domain, fibronectin (FN) III -like domain, and fibrinogen-like domain (Jones FS et al., (2000) Dyv Dyn 208:235-259).
[0003] In early studies, TN-C was considered as an extracellular matrix expressed specifically in cancer stroma. However, recently, it was discovered that a certain amount of expression was found in normal tissues although expression in cancer stroma was extremely high (Kalembeyi I et al., (1997) Int. J.Dev. Biol. 41:569-573). On the other hand, it was revealed that a certain kind of TN-C splicing valiant was expressed specifically in tumor stroma (Jones FS et al., (2000) Dyv Dyn 208:235-259; Borsi L. et al., (1992) Int. J. Cancer 52:688-692; Mighell AJ et al., (1997) Int. J. Cancer 72:236-240). It is known that FNIII domain includes simple 8 (1 – 8) consecutive repeats and 9 spliced repeats (A1 – A4, B, AD2, AD1, C, D) between the 5^{th} and 6^{th} repeats. It is believed that the majority contains insertion of 7 repeats (A1, A2, A3, A4, B, C, D), insertion of a combination of any of the first four, or no insertion. Among them, many cancer tissues including 7 domains are expressed and the last 3 repeats (BCD) are considered relatively specific to cancer stroma. It is believed that clinical applications such as cancer diagnosis are possible by creating monoclonal antibody recognizing this site. There has been no such monoclonal recognizing TN-C valiant only which is expressed specifically to tissue reconstructing site such as cancer stroma.
[0004] Anti TN-C antibody is currently undergoing clinical studies for use in treatment and diagnosis of human glioblastoma (Bigner DD et al., (1998) J. Clin. Oncol. 16:2202-2212; Riva Pet al., (1994) Cancer 73:1076-1082; Riva P et al., (1997) Cancer 80:2733-2742). However, in order to reveal the effectiveness of this antibody in various human diseases, animal test is essential, but the existing antibody does not have cross-reactivity with TN-C of general testing animals such as mouse and rat. Human and mouse are known to have high homology and by creating antibody having cross-reactivity with both human and mouse, it becomes possible to conduct clinical preliminary tests targeting TN-C for human illness in model animals using the same antibody.
[0005]
[Problems to be solved by the invention]

Based on the findings above, the present invention is to provide monoclonal antibody recognizing TN-C splicing valiant expressed specifically in cancer stroma and hybridoma producing the antibody and to contribute this monoclonal antibody for use in medical field.
[0006]
[Means for solving the problems]
The present invention is to provide monoclonal antibody having the recombinant protein of TN-C splicing site expressed specifically in cancer stroma as the antigen and hybridoma producing the antibody. Specifically, the following inventions (1) – (6) will be provided:
(1) An anti-tenascin C monoclonal antibody selectively recognizing tenascin-C splicing variant that is specific to cancer stroma.
(2) A hybridoma cell, FERM P-18070, having a function of producing the said monoclonal antibody.
(3) A hybridoma cell, FERM P-18173, having a function of producing the said monoclonal antibody.
(4) A diagnostic agent characterized in that the said monoclonal antibody is labeled.
(5) A diagnostic agent kit characterized in that it comprises at least both elements of labeled anti tenascin-C secondary antibody for detecting tenascin-C binding to a plate with anti tenascin-C primary antibody fixed thereon as well as to the said primary antibody, and at least one of the said primary antibody or the said secondary antibody is the said monoclonal antibody.
(6) A method of detecting splicing variant that is specific to cancer stroma of the said tenascin C using the said monoclonal antibody.
The embodiments of the present invention will be explained in details below.
[0007]
[Embodiments]
As explained above, tenascin C (TN-C) is a kind of extracellular matrix and some splicing variants are known to exist. Some of them are known to express specifically in cancer stroma. The present invention created a monoclonal antibody using splicing site (BCD) recombinant protein in fibronectin typeIII repeats that is specific to cancer stroma as the antigen. In the specification of this application, the term "tenascin splicing variant that is specific to cancer stroma" refers to the valiant of TN-C including the sites equivalent to the last 3 repeats (BCD) which is relatively specific in cancer stroma in splicing site within the fibronectin typeIII repeats above. The monoclonal antibody of the present invention recognizing such valiant is an effective tool for detecting cancer cells. Also, the expression of such valiant has been observed in patients indicating reconstructing tissues such as inflammations, and therefore, the monoclonal antibody of the present invention is an effective tool for detecting inflammations. Also, as it is indicated in the embodiments below, the monoclonal antibody of the present invention can be used in animal test as it has a cross-reactivity with mouse.
[0008] As indicated in the embodiments below, the applicants used cDNA for coding BCD sites within the fibronectin typeIII repeats of TN-C to collect the fusion protein of the said site, and animals were immunized with the said fusion protein to collect the monoclonal antibody recognizing splicing variant that is specific in cancer stroma. In details, a vector comprising polynucleotide depicted in SEQ ID NO:1 is introduced to Escherichia coli to produce the fusion protein of polypeptide depicted in SEQ ID NO: 2. The fusion protein obtained as above was administered to mouse lacking the gene for TN-C to immunize the said animal using the protein specific to the splicing site. The use of recombinant protein as an antigen makes it possible to produce monoclonal antibody with a clear epitope. When immunization is performed, it is preferred that mouse lacking the gene for TN-C is used in order to prevent the effect of internal TN-C. The use of such mouse lacking the gene makes it possible to obtain a highly reactive monoclonal antibody having cross reactivity.
[0009] A clone of interest can be cloned by preparing splenocytes from immunized mouse, fusing together the splenocytes and mouse myeloma cells and then using limiting dilution method. At this time, it is preferred that HGPRT (hpoxanthin-guanine phosphoribosyl transferase) defective strain with an established method of selecting fused cells is used. Fusing of antibody producing cells and myeloma cells can be performed in for example high concentration polymer solution such as

polyethylene glycol. Furthermore, it is preferred that selection of fused cells are performed using a well-known HAT (hypoxanthine, aminopterin, thymidine) selection method. The HGPRT defective myeloma cells above cannot survive in the absence of aminopterin due to its defective metabolism. However, fused hybridoma cells are able to survive in HAT culture medium as HGPRT is provided from splenocytes and thus are capable of selecting hybridoma from non fused cells. Moreover, cloning is established by removing clones which multiply but do not produce antibody with limiting dilution method.

[0010] The positive clones obtained as above are 4C8 and 19C4 which correspond to hybridoma cells FERM P-18070 and FERM P-18173 respectively. The monoclonal antibody of the present invention can be obtained by culturing and purifying the hybridoma of the present invention using the well-known methods. It is preferred that the culturing of hybridoma cells is performed by culturing in culture medium. Also, hybridoma cells can be injected to mouse intraperitoneally to collect monoclonal antibody from the ascites. The purification of monoclonal antibody obtained as above can be performed using publicly known methods of the field of the invention such as affinity chromatography, ion exchange chromatography, gel filtration method, and ammonium sulfate precipitation It was confirmed that he monoclonal antibody of the presentation obtained as such specifically recognizes cancer stroma as indicated in the embodiments below. It is considered possible to administer drugs efficiently to affected regions by combining for example anticancer drug to the antibody of the present invention because the antibody of the present invention has specific properties.

[0011] The present invention is also a diagnostic agent for cancers and inflammations prepared by labeling a marker to the monoclonal antibody above. Here, enzyme, radioisotope, and fluorescent isothiocyanate can be used as the marker. There is not specific limitation to the enzyme to be used here as long as it fulfills the conditions such as a high turnover number, stable when bound to enzyme, and capable of developing a color by specifically reacting with substrates, and enzyme which is used for normal enzyme immune assays (EIA) can be used. The preferred enzymes include peroxidase, β-galactosidase, alkaline phosphatase, glucose oxidase, acetylcholinesterase, glucose-6-phosphate dehydrogenase, malate dehydrogenase etc. Also enzyme inhibiting substance and coenzyme and the like can be used.

[0012] The bonding of such enzyme and monoclonal antibody can be performed in a publically known method using a cross-linking agent such as maleimide compound. For substrate, a publically known substance can be used in accordance with the enzyme to be used. For example, 3,3',5,5'-tetramethylbenzidine is used when peroxidase is used as the enzyme, or para-nitrophenol is used when alkaline phosphatase is used as the enzyme.

[0013] Radioisotope used as the marker includes the ones used in normal radioimmunoassay such as 125I and 3H. The fluorescent isothiocyanate to be used includes the ones used in a normal fluorescence antibody method such as fluorescence isothiocyanate (FITC) and tetramethyl rhodamine isothiacyanate (TRITC). Also, the present diagnostic agent can be used as immunohistochemistry which is capable of specifically staining tumor stroma. Also, when radioisotope is labeled and administered internally, it can be used to create images of the cancers or inflammations regions.

[0014] The present invention is also a diagnostic agent kit of cancers or inflammations which prepares blood serum from human or animal blood and measures the amount of TN-C protein of splicing variant specific to the cancer stroma in the blood serum. In this method, the TN-C of splicing variant specifically expressed in such lesions can be detected using a so-called sandwich ELISA method (Enzyme-linked immunosorbent assay: enzyme immunoassay method).

[0015] When the diagnostic kit of the present invention is used, first, a sample is placed in contact with the plate on which anti TN-C primary antibody is immobilized to bind the two, and a marker labeled anti TN-C secondary

antibody is coupled to this binary compound. By measuring the signal strength of the marker in the ternary compound, the amount of splicing variant specific to the cancer stroma is measured. Here, as it is indicated in the embodiments below, the monoclonal antibody of the present invention is labeled and can be used as a secondary antibody. It is possible to use the monoclonal antibody of the present invention as the primary antibody for detection with anti TN-C secondary antibody which is labeled its primary antibody.

[0016] The labels of the secondary antibody include various enzyme labels mentioned above in regards to diagnostic agent, that is, peroxidase, β-galactosidase, alkaline phosphatase, glucose oxidase, acetylcholinesterase, glucose-6-phosphate dehydrogenase, malate dehydrogenase etc. Also radiolabels such as 25I and 3H, fluorescent labels such as fluorescence isothiocyanate (FITC) and tetramethyl rhodamine isothiacyanate (TRITC) can be used. When the marker is enzyme, a substrate which develops a color due to an enzymatic activity should be prepared.

[0017] Such diagnostic kits are commercially available in various kinds according to the kind of elements to be tested, and the diagnostic kit of the present invention can be constituted with each element used in publicly known and widely used kits except that TN-C monoclonal antibody is used as the antibody and that anti TN-C antibody is used as the secondary antibody when necessary. Also, it is preferred that a washing fluid for washing unbound TN-C monoclonal antibody and/or unbound secondary antibody is prepared.

[0018] When the diagnostic kit of the present invention is used, the amount of the splicing variant specific to cancer stroma can be measured following the process below.

(1) Immobilize an anti TN-C antibody as a primary antibody on a plate.

(2) Place a sample such as blood serum in contact with the immobilized anti TN-C antibody above and create antigen antibody complex.

(3) Place the said complex in contact with an anti TN-C antibody as a marker-labeled secondary antibody.

(4) Measure the amount of the splicing variant specific to cancer stroma as antigen using the signals from the labels of the secondary antibody.

The present invention will be explained in details using the embodiments below, but the present invention is not limited by the embodiments below.

[0019]

[Embodiments]

Embodiment 1: Preparation of antibody and confirmation of its properties (preparation of immunogen) Polynucleotide (sequence number 1) as cDNA for coding the BCD parts of the fibronectin (FN) III type sequence of TNC was modified to pQE-31 vector and introduced to competent cells (JM-109). This Escherichia coli was incubated, isopropyl-β-D(-) thiogalactopyranoside (IPTG) was added, and incubated overnight to produce fused protein of cDNA comprising pQE-31 vector. After extracting the fused protein by destroying the cell membranes of Escherichia coli, it was purified with a Histrap column (Pharmacia).

[0020] (Preparation of monoclonal antibody) TN-C gene deletion mouse multi–generationally bred at animal testing laboratory at Mie University School of Medicine which are returned to BALB/C and bread were used. Fused protein of BCD in FNIII sequence (prepared in the step above) in the amount of 100μg and the same amount of complete adjuvant were mixed to prepare emulsion and used twice in an interval of 2 weeks in the immunization of mouse. Furthermore, 100μg of fused protein was injected in the tail vein of mouse 3 days prior to cell fusion. Splenocytes was prepared from the spleen collected from immunized mouse, splenocytes and mouse myeloma cells (SP2/0) were mixed at 5:1, and fused using polyethylene glycol (PEG).

[0021] Then, PEG was removed and resuspended in IMDB containing 5% of Briclone (Bio Research) and 20% FCS, and plated in microplate at the concentration of 1 x 105/ hole, and incubated at CO_2 7%, 37°C. HAT medium culture was added 4 days later and ELISA was performed 7 – 10 days later using purified TN-C derived from human glioma as

antigen and antibody production was confirmed. Clone selectively incubates using HT culture medium and cloning was repeated twice using limiting dilution method. Moreover, using mouse fetus section and human breast cancer tissues, usable clones were immunohistochemically selected. The isotype of monoclonal antibody was determined using Mouse monoclonal antibody isotype kit (Amersham LIFE SCIENCE). Also, for comparison, 4F10, which was previously prepared by the applicants, was used as the antibody recognizing all valiant of TN-C.

[0022] (Purification of antibody) Antibody producing cells were incubated in a serum-free culture medium, GIT culture medium (Wako), and antibody was produced until 80% of the cells were dead. After removing cells by centrifugation (1000rpm, 15min), it was adjusted to ammonium sulfate at 50% saturated condition, let stand overnight at 4°C, and the depositions were collected by centrifugation (1000rpm, 30min). The depositions were dissolved in binding buffer (protein AMAPS II kit) which has been diluted twice, and IgG was absorbed to protein A column (Pharmacia-Amersham). Subsequently, it was PBS-dialyzed overnight.

[0023] Using the TN-C purified from human glioblastoma culture supernatant as the antigen, clones producing antibody reacting to native TN-C were screened using ELISA method, and 13 clones with high antibody values were selected. For the 13 clones, in order to investigate the reactivity against TN-C in mouse and human tissues, immunohistochemical staining was performed using paraffin sections of mouse fetus tissues and human breast cancer tissues and 2 clones with high reactivity in both were selected (4C8, 19C4). The isotypes of immunoglobulin of such clones were IgG1, κ.

[0024] Between 2 clones (4C8, 19C4), the hybridoma α-hTNC/B-D for 4C8 was deposited with deposition number FERM P-18070 at National Institute of Advanced Industrial Science and Technology on October 4, 2000. Also, the hybridoma α-hTNC/B-D/2 for 19C4 was deposited with deposition number FERM P-18173 at Natl. Inst. of Biosciences and Human-Technology of the Agency of Industrial Sci. and Technology on January 23, 2001.

[0025] (Western blotting) Next, in order to confirm that such antibodies recogniz splicing site of TNC, purified human TN-C was electrophoresed and western blotting was performed. At this time, antigen (4F10) which recognizes all valiant of human TN-C was used as a reference. Human TN-C was purified from cell strains of Human Glioma Cells by salt deposition using ammonium sulfate at 50% saturated condition, Sephacryl S500 column, and mono Q column (Pharmacia Biotech).

[0026] Electrophoresis (SDS-PAGE method) was performed with Laemmli method on Polyacrylamide Gel Electrophoresis MULTIGE L2/15 (Daiichi chemical). The electrophoresed protein was electrically copied to Immobilon Transfer Membranes (Millipore) The membrane was blocked by 50mMTris-buffered saline (TBS) containing 0.5% skim milk and reacted overnight with monoclonal antibody which has been diluted 4000 times with TBS containing 0.1% skim milk, washed with 0.1% skim milk solution, diluted 500 times with peroxidase label goat anti-mouse IgG (H+L chain) (Biorad) and reacted as the secondary antibody, washed, and developed color in DAB/H2 O2 solution.

[0027] The results are indicated in Fig. 1. A wide band was recognized from 200 – 300kDa in 4F10 and especially a dark band was observed around 210kDA and 300kDa. On the contrary, a dark band was confirmed around 300kDa in 4C8 and 19C4, but no band was observed around 210kDa. That is, a low molecular valiant was not recognized in 4C8 and 19C4 and only high molecular weight band was recognized. The band around 210kDa is considered as TN-C without the insertion at the splicing site and the band around 300kDa is considered as the valiant with the insertion of seven FNIII repeats. Thus, 4C8 and 19C4 were not able to recognize TN-C around 210kDA but were able to recognize TN-C containing repeats which are normally spliced.

[0028] (Immunohistochemical staining using frozen mouse sections) In order to confirm the cross-reactivity with mouse, frozen sections of mouse breast cancer tissues were used for immunohistochemical staining. Frozen

sections were prepared from mouse breast cancer tissues, air dried, and immobilized for 10 minutes using 10% formalin. After repeating PBS wash 3 times, normal goat blood serum which has been diluted 10 times with PBS was used for blocking 30 minutes. As the primary antibody, sections were reacted for 2 hours with FITC labeled TN-C monoclonal antibody (4C8, 19C4, and 4F10) which has been diluted 20 times with PBS, washed with PBS and sealed. It was then observed using a epifluorescence microscope with a filter suitable to FITC and pictures were taken. The results are indicated in Fig. 2. The breast cancer stroma was stained stronger in 4F10 and 4C8. Although it was weak, 19C4 showed staining properties.

[0029] Embodiment 2: In order to investigate whether or not TN-C containing splicing sites which recognizes 4C8 with immunohistochemical staining in human tissues are specifically expressed in cancer stroma, immunohistochemical staining was performed using paraffin sections of human tissues. Among the prepared antibodies, 4C8 clone antibody was used for immunostaining. The paraffin sections of human tissues obtained from autopsy was hydrated with ethanol series after paraffin was removed using xylene. Endogenous peroxidase was inactivated by being submerged in -.3% H_2O_2/ methanol solution for 10 minutes. Antigenicity was activated by pepsination for 4F10 antigen as the primary antibody and by 0.1% saponin for 4C8 antibody at room temperature for 20 minutes. After repeating PBS wash 3 times, normal goat blood serum which has been diluted 10 times with PBS was used for blocking 30 minutes. Subsequently, using 4F10 and 4C8 which has been diluted 200 times as the primary antibody were reacted overnight. Labeling was performed using scytek polyvarent HPR kit (SCYTEK) and color was developed using DAB/H_2O_2 solution, and then stained using hematoxylin for 15 seconds to make a permanent preparation.

[0030] The sample tissues included human lacteal gland (breast cancer), esophagus, stomach, small intestine, spleen, cardiac muscle, kidney, liver, lung, tracheal cartilage and adrenal gland. For comparison, immunohistochemical staining was performed on 4F10 as a primary antibody which recognize all variant as reference. The results are summarized in Table 1.

[0031]

[Table 1]

Organs	Tissues	4F10	4C8				
Lacteal gland				Lungs			
	Brest cancer stroma	++	++		Alveolar walls	+	-
	Normal lacteal gland duct	+	-	Liver			
Small intestine					Sinusoidal wall	+	-
	Basement membrane of the epithelium	+	-		Central vein	+	-
	Muscularis mucosae	+	±	Adrenal gland			
	Muscular layer	+	±		Marrow	+	-
Esophagus					Cortex	+	-
	Basement membrane of the epithelium	+	-	trachea			
	Muscularis mucosae	+	-		Cartilage membrane	+	-
	Muscular layer	+	-		Cartilage cells	+	-
	Around esophageal glands	+	-	Blood vessel			
Stomach					Muscular wall of vessels	+	-
	Basement membrane of the epithelium	+	±		Capillary walls	+	-
	Muscularis mucosae	+	-				
	Muscular layer	+	-	Cardiac muscle		-	
Kidney				Spleen		-	
	Medullary interstitium	+	+				
	Renal cortical interstitial tissue	+	-				

[0032] Fig. 3 indicates the results of immunohistochemical staining of various human tissues. In Fig. 3, a and b, c and d, e and f, g and h display the results in lacteal gland tissue, small

intestine mucus membrane, renal medullary, and tracheal cartilage respectively. The used antibodies are 4F10 antibody and 4C8 antibody for a, c, e, g and b, d, f, h respectively. In lacteal gland tissues, 4C8 strongly stained stroma of tumor tissues and the remaining normal breast cancer tissues did not display any positive images (Fig. 3b). Staining was not observed in other tissues except for a weak staining in renal medullary stroma (Fig. 3f) and muscular wall of the alimentary canal (Fig. 3d).

[0033] In lacteal gland tissues, 4F10 expressed highly in tumor stroma and staining was also observed in the basement membrane of the normal lacteal gland duct (Fig. 3a). Also in other normal tissues, the basement membranes of mucosa of the gastrointestinal tract (Fig. 3c) or renal tubular epithelium and the stroma of renal medullary were stained (Fig. 3e). Also, positive images were observed among smooth muscle cells of muscularis mucosae as well as longitudinal layer, and on muscular wall of vessels of arterioles. Also, normal basement membrane of capillary, sinus lienis, sinusoid, and alveolar walls were stained well. The staining of capillary walls was observed in zona fasciculate and dictyosome of the adrenal cortex. Also, in tracheal cartilage, cartilage membrane and cartilage cells were stained.

[0034] Embodiment 3: TN-C recovery test by ELISA method

In a multi-well plate having 96 holes, 4F10 antibody which has been diluted 500 times with 100μl of phosphate buffer saline solution was added and let stand at 4°C overnight to absorb antibody. The antibody solution was removed, blocked with 1% bovine albumin (BSA), and washed with Tris buffer saline solution (TBS). Using the TN-C purified from culture supernatant of human glioblastoma cell U251as the reference standard, serial 1:3 dilution of 1% BSA was performed and placed in a 100μl/ well. Also, TNC reference standard was added using serial dilution to the human normal blood serum which has been diluted 2 times with 1%BSA to perform a recovery test. TN-C diluted solution was added, let stand at room temperature for 2 hours, washed with TBS, peroxidase standard anti TNC antibody (19C4) Fab' was added, and let stand at room temperature for 1 hour. Subsequently, it was washed with TBS, developed color using o-phenylenediamine as the coloring agent, and measured with a microplate reader.

[0035] As a result, human normal blood serum which has been 2 times diluted recovered 64ng/ml of TN-C and this value was within the normal range in other human blood serum. According to the added amount, increase along the standard curve measured by the reference standard (Fig. 4) was confirmed, and the added TN-C was well recovered. In Fig. 5, X indicates standard curve and ○ indicates the result of recovery test.

[0036] Embodiment 4: TN-C measurements in the blood of patient with myocardial infarction using ELISA method

Among the blood serum collected over time from 8 cases of patient with myocardial infarction, TN-C was measured 3 times at administration to the hospital, a couple of days later (2 – 4 days), and 1 week later. The blood serum was diluted 4 times and measured using the same ELISA method as the recovery test above. The standard curve was made using purified human TN-C as the reference standard.

[0037] In the blood serum of patient with myocardial infarction, it reached the highest value a couple of days after the administration to the hospital and 1 week later, it went down to the same level as administration to the hospital. The results are indicated in Fig. 5. In the histological studies of test animals, it is know that TN-C expresses in early stage after cardiac muscle involvement, and the expression hit the highest a couple of days later, and then the expression decreases 1 week later. The TN-C concentration in blood serum is considered to be reflecting such tissue expression. From this result it was discovered that the monoclonal antibody of the present invention is not only capable of measuring TN-C in blood serum from diseased tissues, but also capable of detecting high-molecular TN-C expressed inflammation changes and tissue reconstructing process after tissue necrosis.

[0038]

[Advantages of the invention]

To provide a monoclonal antibody that recognizes TN-C splicing variants specifically expressed in cancer tissues and inflammation tissues and hybridoma cells producing the said antibody. The monoclonal antibody according to the present invention is useful for diagnosing cancers and inflammations.

[0039]
[Sequence listing]

<110>Name of applicant: President of Mie University
<120>Title of the invention: Anti-tenascin C monoclonal antibody and hybridoma producing the same
<160>Number of sequence: 2
<210>Sequence number: 1
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<160>Type of sequence: Nucleic acid
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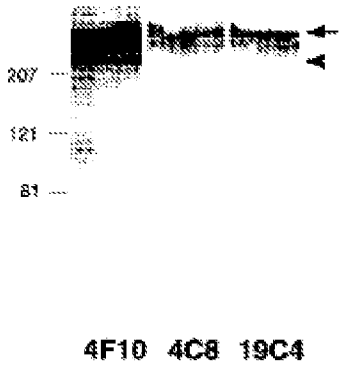
SerGlyPheThrGlnGlyHisGlnThrLys ProLeuArgAlaGluIleValThrGluAla 240
GluProGluValAspAsnLeuLeuValSer AspAlaThrProAspGlyPheArgLeuSer 260
TrpThrAlaAspGluGlyValPheAspAsn PheValLeuLysIleArgAspThrLysLys 280
GlnSerGluProLeuGluIleThrLeuLeu AlaPro 292

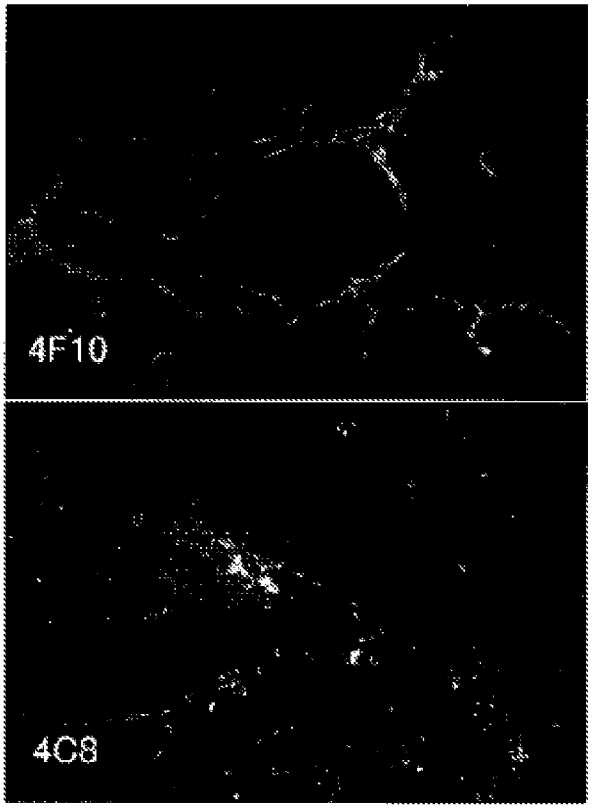
[Brief description of the drawings]
[Fig. 1] Fig. 1 is a picture of western blotting showing that the anti-TN-C antibodies (4C8, 19C4) of the present invention recognize high-molecular valiant.
[Fig. 2] Fig. 2 is a picture showing the results of performing immunohistochemistry on frozen sections of mouse breast cancer tissues using anti TN-C antibody (4F10) as a reference as well as the anti TN-C antibody (4C8) of the present invention.

[Fig. 1]

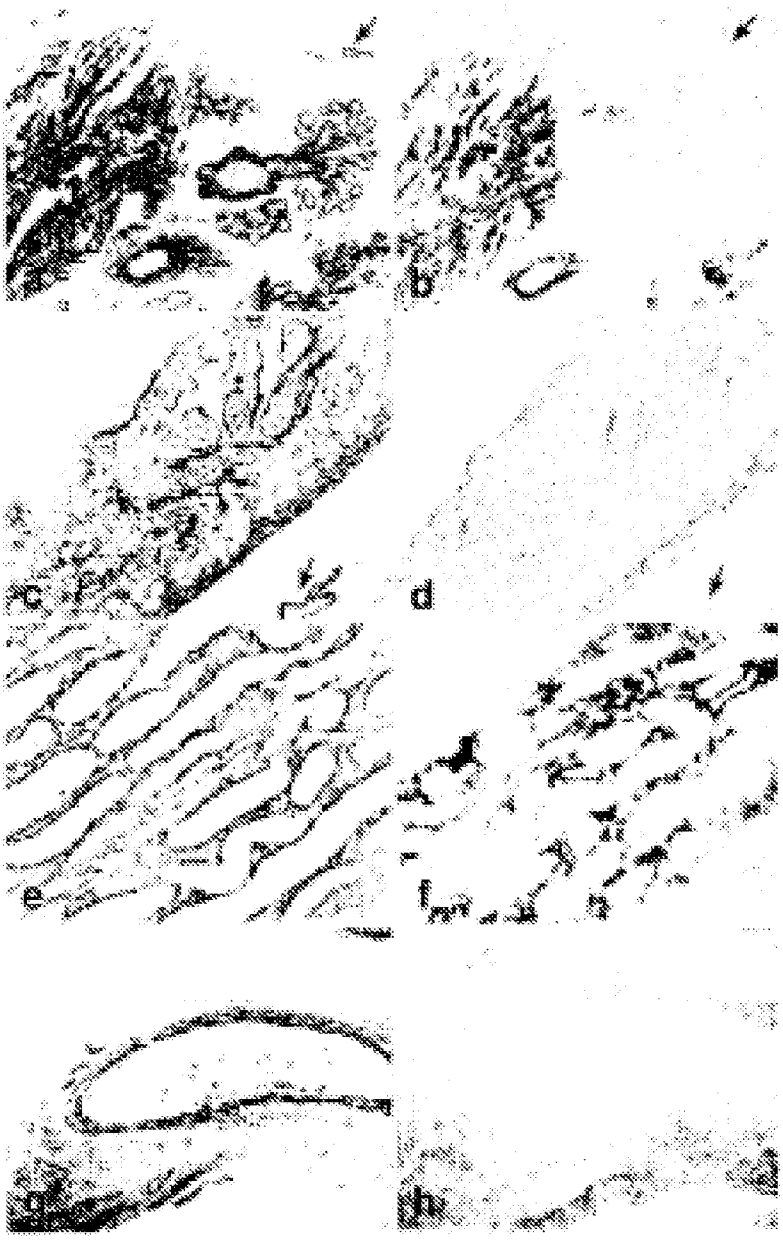


[Fig. 3] Fig. 3 is a picture showing the results of performing immunohistochemistry on various human tissues using anti TN-C antibody (4F10) as a reference as well as the anti TN-C antibody (4C8) of the present invention.
[Fig. 4] Fig. 4 is a chart indicating the reference curve created by ELISA of anti-TN-C antibody and the results of recovery test
[Fig. 5] Fig. 5 is a chart measuring the time-dependent change of TN-C concentration in blood serum from patient with myocardial infarction.

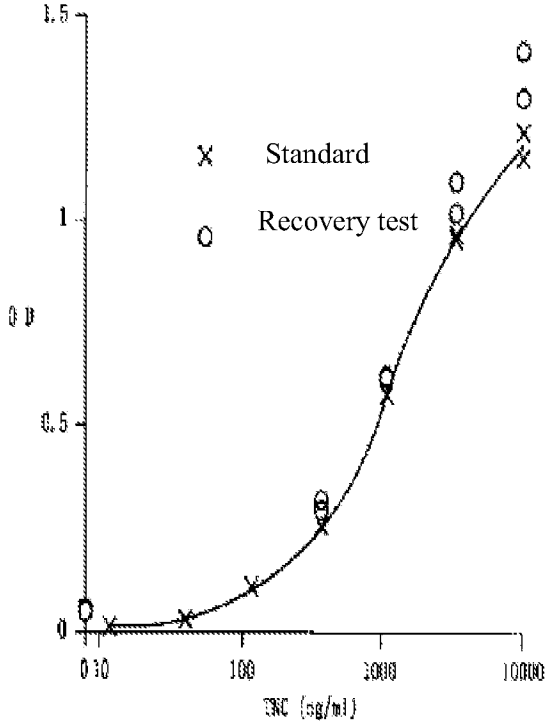
[Fig. 2]



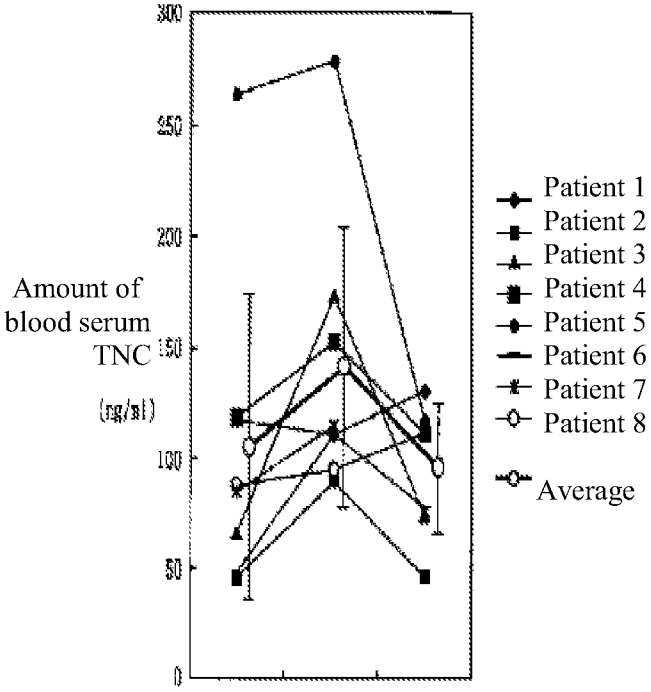
[Fig. 3]



[Fig. 4]



[Fig. 5]



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